Review article

Fenfen Zheng*, Weiwei Xiong, Shasha Sun, Penghui Zhang and Jun Jie Zhu*

Recent advances in drug release monitoring

https://doi.org/10.1515/nanoph-2018-0219
Received December 14, 2018; revised February 1, 2019; accepted February 2, 2019

Abstract: Monitoring drug release in vitro and in vivo is of paramount importance to accurately locate diseased tissues, avoid inappropriate drug dosage, and improve therapeutic efficiency. In this regard, it is promising to develop strategies for real-time monitoring of drug release inside targeted cells or even in living bodies. Thus far, many multi-functional drug delivery systems constructed by a variety of building blocks, such as organic molecules, polymeric nanoparticles, micelles, and inorganic nanoparticles, have been developed for drug release monitoring. Especially, with the advancements in imaging modalities relating to nanomaterials, there has been an increasing focus on the use of non-invasive imaging techniques for monitoring drug release and drug efficacy in recent years. In this review, we introduce the application of fluorescence imaging, magnetic resonance imaging (MRI), surface-enhanced Raman scattering (SERS), and multi-mode imaging in monitoring drug release, involving a variety of nanomaterials such as organic or inorganic nanoparticles as imaging agents; their design principles are also elaborated. Among these, a special emphasis is placed on fluorescence-based drug release monitoring strategies, followed by a brief overview of MRI, SERS, and multi-mode imaging-based strategies. In the end, the challenges and prospects of drug release monitoring are also discussed.

Keywords: drug delivery system; drug release monitoring; fluorescence imaging; MRI; SERS; multi-mode imaging; nanomaterials.

1 Introduction

In the realm of inoperable cancer-based therapies, a number of therapeutics (e.g. drugs, antibodies, gene strands) are highly recommended for inhibiting tumor growth and preventing recurrence. However, due to the poor selectivity and high toxicity, their severe side effects to normal organs and limited therapeutic efficacy to tumor tissues have hindered their clinical usage for cancer therapy [1–4]. In recent years, the flourishing development of chemistry and material science has brought about a burst of research activities in drug delivery systems (DDSs), which are aimed to address the biomedical challenges in traditional medicine. To achieve site-specific drug delivery for controllable pharmacokinetics and biodistribution, a variety of targeted DDSs were designed. The most common strategy to achieve high targeting efficiency is based on passive targeting, which involves the enhanced permeability and retention (EPR) effect caused by the leaky vasculature and dysfunctional lymphatic drainage of many solid tumors [5, 6], usually resulting in the high accumulation of macromolecules as well as nanoparticles with a diameter of 20–500 nm in the tumor tissue [7 , 8]. This targeting strategy is universal and enables the DDS to treat a wide range of solid tumors. Differently, active targeting relies on specific interactions between membrane-bound receptors (e.g. proteins, glycoproteins, and lipoproteins) on tumor cells and corresponding ligands (e.g. saccharides, lectins, antibodies and antibody fragments, peptides, nucleotides, enzymes, or enzyme inhibitors) on the DDS surface. The targeting ligands can guide the delivery vehicle to the desired action site and avoid non-specific uptake by healthy cells and tissues [9, 10]. The active targeting strategies are not only selective and effective against solid tumors, but they also show potent efficacy to leukemia and circulating tumor cells that are less sensitive to passively targeted DDSs. To further enhance the specific drug release at the tumor site and improve the therapeutic efficacy, controllable
DDSs were developed to respond to specific stimuli [11, 12], including exogenous triggers (e.g. temperature [13], magnetic field [14, 15], ultrasound [16], light [17, 18], electric pulses [19, 20], etc.) and endogenous triggers (acidic pH [21, 22], enzyme [23, 24] or redox species [25, 26], etc.), making it possible to deliver drugs in spatial-, temporal- and dosage-controlled fashions. However, in most cases, crucial data of in situ and real-time drug release from these DDSs in living cells or in vivo cannot be directly obtained, and the unknown actual drug concentrations at the focus have perplexed doctors. To overcome these hurdles, multi-functional DDSs, capable of targeting tumor cells, controlling drug release, and real-time monitoring, are being developed, which is absolutely critical when an on-demand dose of drug is needed to reach the pathological region of interest without damaging normal tissues.

As is known, except for serving as promising scaffolds for drug loading, most organic and inorganic nanomaterials with unique physicochemical properties have been applied as imaging contrast agents for early disease detection and treatment monitoring [27–29]. After being integrated with a tracking method, the multi-functional DDS can not only image the targeted tissues but also monitor the drug release kinetics in a real-time and long-term fashion. Considering the limitations of invasive imaging in clinical treatment, non-invasive imaging techniques, such as fluorescence imaging, magnetic resonance imaging (MRI), and surface-enhanced Raman scattering (SERS) imaging, are well developed for monitoring drug delivery, drug release, and therapeutic efficacy [30–33]. Moreover, to optimally integrate the advantages of different imaging modes in one DDS, multi-mode imaging (allowing more than one imaging mode to act synergistically) was also established. These real-time systems for tracking where, when, and how drugs are delivered and released may provide an in-depth insight into the drug uptake process, pharmacokinetics, biodistribution, local distribution at the target site, kinetics of drug release, and therapeutic efficacy. In turn, this spatiotemporal information on the DDSs and drugs also offers guidance for the development and optimization of drug therapy in terms of DDS engineering, drug release modalities, and administration approaches.

In this review, we provide an overview of recent advances in drug release monitoring followed by highlights of four imaging strategies in the field of drug release monitoring: fluorescence imaging, MRI, SERS, and multi-mode imaging. In each case, we present the merits of the imaging technique as well as the advances of nanomaterials that enable the technique to overcome the barriers for drug release monitoring and effective drug therapy. Among them, we mainly introduce the development of the fluorescence-based drug release monitoring strategies and briefly outline the MRI, SERS, and multi-mode imaging-based strategies. We hope that this review will provide the most updated summary of the design, performance, and application of drug release monitoring strategies.

2 Fluorescence-based drug release monitoring strategies

Fluorescence is produced by the radiative transition of excitation energy after light absorption, which is fast, with a lifetime decay in nanoseconds. Among the biomedical imaging technologies, fluorescence imaging can timely reflect the biodistribution and pharmacokinetics of DDSs due to its real-time, non-invasive, highly sensitive, and radiation-free features [34–36]. To track drug transportation and release in vitro and in vivo, efforts have been devoted to couple fluorescent agents to the drug release event in a delivery system, and real-time information about the release process can be obtained using non-invasive fluorescence imaging techniques. Particularly, among various fluorescence imaging systems, near-infrared (NIR) fluorescence imaging has become the overwhelming choice to visualize species of interest in vivo, because NIR protons can deeply penetrate tissue and skin with minimal background interference [37, 38]. Moreover, multiple fluorophores can be imaged in the same cell or animal, facilitating the development of multi-channel imaging for tracking multiple biological events simultaneously. As the performance of fluorescence-based drug release monitoring strategies greatly depend on the optical property of imaging agents as well as the nanocarriers, in this section, various organic or inorganic fluorescent agents and carrier materials are summarized and discussed.

2.1 Prodrug-based drug release monitoring

Over the past decades, prodrugs were developed as an effective approach for cancer therapy. This strategy provides great possibilities to overcome various drawbacks of drug molecules, such as poor solubility, systemic instability, and lack of cancer specificity. Recently, theranostic prodrugs, which exhibit the dual purpose of diagnosis and therapy, are emerging as a promising technology in biomedicine. The theranostic prodrugs can covalently bind with fluorophores as fluorescent reporters to monitor the
drug release process, and the fluorescence signals could be of great benefit to evaluate their therapeutic effect, pharmacodynamics, and activation mechanism. These prodrugs enable researchers to know when, where, and how the active drug molecules are delivered, and can even semi-quantitatively detect the release of active drugs in a non-invasive manner.

By coupling latent fluorophores to the drug molecules with an activatable linker, real-time information about the drug release process can be obtained by using non-invasive fluorescence detection techniques. For instance, Weinstain et al. [39] reported a DDS for real-time monitoring drug release in vitro by using a coumarin-based linker. In this system, the drug release event occurring in the cytoplasm of human umbilical vein endothelial cells was observed directly with the aid of confocal microscopy, and the amount of released drug was calculated by quantifying the emitted fluorescence, allowing the prediction of therapeutic effect and potential side effects. Similarly, Bazylevich et al. [40] developed a fluorescein-based prodrug by conjugating free phenolic hydroxyl of fluorescein chemosensor to the hydroxyl group of the drugs for monitoring drug release. Hu and Zeng [41] designed a thienoacridine prodrug, composed of an anti-cancer drug camptothecin (CPT), a cleavable linker based on disulfide bonds, and a fluorophore naphthalimide derivative, and detected the drug release via the fluorescence resonance energy transfer (FRET) mechanism. The FRET occurring between CPT (energy donor) and naphthalimide (energy receptor) was interrupted by the high concentration of intracellular glutathione (GSH), thus achieving dual fluorescence response and specific release of CPT. Liu et al. [42] reported a ratiometric thiorphan prodrug consisting of boron dipyrromethene, therapeutic drug CPT, and a long flexible disulfide linker. Once treated with dithiothreitol, the disulfide bond was cleaved and CPT was released, resulting in enhanced blue and green fluorescence emissions. The changes of the fluorescence ratio over time provided the opportunity for the real-time monitoring of drug release at cellular levels. DT-diaphorase, an enzyme overexpressed in some malignant tumors, can be exploited to catalyze the reduction of quinine propionic acid moiety incorporated into prodrug, which could be utilized for concomitant drug release imaging and cancer chemotherapy [43, 44]. In order to monitor drug release more accurately and understand pharmacological mechanisms better, Kong et al. [45] developed a dual turn-on fluorescence based controlled release system (CDox), in which doxorubicin (Dox) and fluorescent dye (CH) are conjugated by a pH-responsive cleavable hydrazine linker. The C=N isomerization and N-N free rotation efficiently quenched the fluorescence of CH and Dox in the CDox complex. When activated under acidic conditions, CDox was hydrolyzed to release Dox and CH, resulting in dual turn-on signals with emission peaks at 595 and 488 nm, respectively. Using this system, two-photon fluorescence imaging of CDox in living tumor tissues was successfully performed by high-definition three-dimensional (3D) imaging.

NIR fluorophores are often used as fluorescent reporters for in vivo drug release monitoring because NIR photons can deeply penetrate the skin and underlying tissue with minimal damage to the biological samples and with low background interference. Zhang et al. [46] reported a real-time drug-reporting conjugate (CPT-SS-CyN), composed of a NIR fluorescent cyanine-amine dye (CyN), a disulfide linker, and a model therapeutic drug (CPT) (Figure 1A). Likewise, treatment with dithiothreitol induces cleavage of the disulfide bond, resulting in the release of NIR dye and the drug CPT. The released CyN has an emission wavelength (760 nm) that is significantly different from that of CPT-SS-CyN (820 nm), enabling easy detection and monitoring of drug release. A linear relationship of the fluorescence intensity at 760 nm versus the amount of released CPT was observed, enabling quantitative monitoring of drug release in live cells and semi-quantitative monitoring in live animals in a real-time and non-invasive manner. Additionally, a NIR fluorophore, dicyanomethylene-4H-pyran derivative (DCM), was conjugated with anti-cancer CPT through a disulfide linker (DCM-S-CPT), which can be cleaved by the overexpressed intracellular GSH, making the drug activation and fluorescence turn on concomitantly. Furthermore, DCM-S-CPT has been successfully utilized for assessing drug release and cancer therapeutic efficacy by analyzing NIR fluorescence in vivo and in situ (Figure 1B) [47].

The fluorescent signals of single-channel fluorescent theranostic prodrugs were usually unobservable before drug release; therefore, it is difficult to simultaneously monitor their biodistribution and drug release in spatiotemporal mode. The metabolism kinetics of prodrugs in a certain organ or tissue becomes a blind spot, which is a critical obstacle in the precise diagnosis and chemotherapy. The development of two-color imaging systems with two-channel fluorescence that can distinguish the signals before and after drug release has opened a window to overcome this hurdle. Liu et al. [49] reported a novel light-activated cleavable prodrug comprising a photoremovable coumarinyl, the anti-cancer drug CPT, a cleavable linker, and the NIR fluorescent dye DCM. In this prodrug, owing to FRET, the fluorescence of coumarinyl and CPT is completely quenched while the DCM moiety shows red emission, which
can be used to visualize the drug localization before irradiation. Upon one- or two-photon irradiation, the prodrug releases active CPT, and the release process is monitored via the change in the FRET process. Ye et al. [48] presented a dual-channel NIR activatable theranostic prodrug, in which the CPT was linked to a NIR cyanine dye via a disulfide linkage (Cy-S-CPT) (Figure 1C). The cleavage of the disulfide bond by endogenous GSH activated the anti-cancer drug
and induced a remarkable fluorescence shift from 825 to 650 nm, thereby providing dual fluorescent channels to real-time track the prodrug biodistribution and activation in vivo. As demonstrated, the prodrug was activated in all organs, particularly in the liver after an intravenous injection, and achieved predominant accumulation and activation in tumors at 24 h after injection.

In addition, such a dual-channel fluorescence strategy can also be applied to monitor drug release and evaluate therapeutic efficacy simultaneously. As is known, the activity of intracellular apoptotic proteases such as caspase-3 can reflect the degree of cell apoptosis, which is highly related to the therapeutic efficacy. Based on these, Li et al. [50] developed a versatile prodrug for real-time drug release monitoring and in situ therapeutic efficacy evaluation. In this system, the fluorescence of both 5(6)-carboxyfluorescein and Dox was quenched by 4-(dimethylaminoazo)benzene-4-carboxylic acid via FRET. On the one hand, the acid-mediated Dox release from the prodrug was real-time monitored according to the increase of liberated Dox fluorescence. On the other hand, Dox-induced cell apoptosis was in situ assessed by the fluorescence recovery of 5(6)-carboxyfluorescein, resulting from the caspase-3-mediated cleavage of the Asp-Glu-Val-Asp peptide. Through this cascaded imaging strategy, the drug release and consequent cell apoptosis were real-time monitored, enabling the in situ detection of pharmacokinetics and treatment response.

### 2.2 Macromolecule-based drug release monitoring

As driven by the exponentially increasing demand for “intelligent” materials, responsive polymers, polymeric nanoparticles, micelles, and other macromolecules that are capable of adapting to changes in local environment have attracted tremendous interest in recent years. These materials with autofluorescence or labeled fluorescence have been exploited for controlled drug release with real-time tracking ability due to their low cytotoxicity and good biocompatibility.

Most of the anti-cancer drugs have little aqueous solubility and permeability. One common way to deliver hydrophobic drugs to the target site is to construct a prodrug that can create a synthetic analogue with higher hydrophilicity by modification. However, even with this approach, it is still difficult to extend the circulation time of drugs in the blood and to suppress the fast renal excretion, thus lowering the curative effect. One way to solve this problem is incorporating the drug molecules with polymer nanoparticles. Wu et al. [47] and Ye et al. [48] demonstrated that loading the prodrug into polymeric nanoparticles could significantly improve the therapeutic efficacy and lower the side effects owing to the EPR effect. Another example is perylene, which can act as an efficient phototrigger for carboxylic acids and alcohols in aqueous media under visible light irradiation. The perylene molecules can form nanoparticles with different sizes by the re-precipitation method, and the perylene nanoparticles show different spectral properties compared with the free perylene molecules. Jana et al. [51, 52] reported chlorambucil-conjugated perylene organic nanoparticles as single-component photosensitive nanocarriers for controlling and monitoring drug release. In these systems, perylene nanoparticles performed four roles: (i) nanocarriers for drug delivery, (ii) phototriggers for drug release, (iii) fluorescent chromophores for cell imaging, and (iv) detectors for real time-monitoring of drug release.

Drug molecules can also conjugate with polymer nanocarriers to improve their efficacy and toxicity. The conjugated drugs are typically inactive in the polymer nanocarriers, and the triggered release will recover their therapeutic activity. To monitor the drug release in the polymer-drug conjugates, Shiran et al. [53] designed two N-(2-hydroxypropyl) methacrylamide co-polymer-based systems that were complementary to one theranostic nanomedicine consisting of self-quenched Cy5 as a reporter probe and paclitaxel as an anti-cancer agent. The established co-polymer systems enabled site-specific release upon enzymatic degradation in cathepsin B-overexpressed breast cancer cells, and the release of paclitaxel occurred concomitantly with the activation of Cy5 to the turn-on state. Apart from conjugating additional moieties for nanoparticle assembly, some drug molecules can self-assemble into nanoparticles for delivery. Zhang et al. [54] developed a self-carried curcumin nanodrug for highly effective cancer therapy in vitro and in vivo with real-time monitoring of drug release. Curcumin has different fluorescence characteristics in its molecular (“on” state, strong green fluorescence) and solid (“off” state, no emission because of intermolecular aggregation) forms, which was exploited in this study for monitoring the release of curcumin molecules (“on”) from drug nanoparticles (“off”) upon cell internalization.

Polymer nanoassemblies, such as polymer micelles, polymer vesicles, and polyionic complexes, are commonly created from self-assembling or cross-linked co-polymers. They are typically engineered to have a hydrophobic core to entrap drugs with low solubility and an enveloped hydrophilic shell to protect the drugs from precipitation, degradation, or non-specific binding to plasma proteins. By virtue of physically encapsulated or covalently linked
fluorophores, polymeric nanoassemblies have been extensively explored for tracing the biodistribution of nanocarriers and monitoring the drug release. For example, a series of fluorescent polymeric micelles have been constructed by self-assembly of two amphiphilic co-polymers with fluorescent moieties based on different fluorine derivatives. The drug release from these polymeric micelles could be monitored by the on/off of FRET from micelles to drugs [55]. Specifically, the modulating environment of some polymer nanoparticle cores, such as hydrophobicity or pH sensitivity, can control their fluorescence property. By tethering hydrophilic polymer chains (poly(ethylene glycol), PEG) onto a single polymer backbone (branched poly[ethylene imine], PEI) while modifying the core of the nanoassemblies with lipophilic pendant (palmitate, PAL) to modulate fluorochromism of Nile blue (NB), two types of polymer nanoassemblies were synthesized: PEG-PEI-NB (2PN) and PEG-PEI-PAL-NB (3PN) [56]. 2PN has ionizable moieties in the core that can attract water and quench NB fluorescence, while 3PN has a hydrophobic core modified with PAL to increase entrapment of hydrophobic anti-cancer drugs. Therefore, fluorescence intensity increased upon addition of hydrophobic drugs or exposure to acidic environments. These two polymer nanoassemblies were used to monitor the amount of drug remaining in the hydrophobic core in real time and detect metastatic tumors in a tissue monitoring the amount of drug remaining in the hydrophobic environments. AIE micelles prepared by conjugation of TPE with PEG onto a single polymer backbone (branched poly[ethylene imine], PEI) while modifying the core of the nanoassemblies with lipophilic pendant (palmitate, PAL) to modulate fluoro- chromism of Nile blue (NB), two types of polymer nanoassemblies were synthesized: PEG-PEI-NB (2PN) and PEG-PEI-PAL-NB (3PN) [56]. 2PN has ionizable moieties in the core that can attract water and quench NB fluorescence, while 3PN has a hydrophobic core modified with PAL to increase entrapment of hydrophobic anti-cancer drugs. Therefore, fluorescence intensity increased upon addition of hydrophobic drugs or exposure to acidic environments. These two polymer nanoassemblies were used to monitor the amount of drug remaining in the hydrophobic core in real time and detect metastatic tumors in a tissue pH-dependent manner. Aibani et al. [57] exploited differences in the hydrophobic/hydrophilic balance between two isomers, spiropyran and merocyanine, to mediate drug release from a polymer micelle. The stable spiropyran with closed hydrophobic four-ring system preferentially favors a non-polar environment, whereas light irradiation can convert it to its zwittrionic merocyanine counterpart with open ring, which prefers a more hydrophilic environment. In addition, the drug was quantified using a FRET pair between spiropyran-merocyanine photochromic dyad and co-encapsulated boron dipyrromethene fluorophore. Therefore, this DDS based on polymer micelles not only is capable of releasing cargo under light, but also provides a real-time analysis of unreleased cargo.

The aggregation-induced emission (AIE) molecules, such as tetraphenylethene (TPE), triphenylethene, and siloles, commonly show no emission in dispersed solution due to the decaying of the excited energy via intramolecular rotations, while they exhibit high emission at the aggregated state because of the restricted intramolecular rotations. Such AIE phenomena can also be observed when the AIE molecules are aggregated in body fluids or in solid state, which favors cell imaging, chemical sensors, and investigating drug delivery mechanisms. For instance, AIE micelles prepared by conjugation of TPE with PEG were used as both nanocarriers to deliver anti-cancer drug and intracellular imaging probe to track their locations. Liow et al. [58] constructed a thermogelling co-polymer, poly(PEG/PPG/TPE urethane), consisting of hydrophilic PEG segment, hydrophobic poly(propylene glycol) (PPG) segment, and TPE units with AIE character (Figure 2A). As the AIE of the AIE thermogel is highly dependent on the Dox concentration and temperature, the drug release status and the erosion condition of the matrix can be revealed. Similarly, the PNIPAM thermogel can combine with LaF₃:Eu³⁺ nanocrystals by surface-initiated living radical polymerization [61]. The fluorescence properties and drug release behaviors in the core-shell LaF₃:Eu³⁺ nanocrystals/PNIPAM nanogels are highly related to the temperature. The fluorescence intensity of the nanogels increases gradually with the drug release.

Further, coordination polymers comprising organic ligands and metal ions can also be used for efficient monitoring of drug release in tumors. Fe²⁺ and polyphenol gallic acid can form a polygonal nanoscale coordination polymer with extremely high Dox loading efficacy (up to 48.3%) in aqueous solution, which exhibits potent anti-tumor effect [62]. In vitro studies demonstrated that the Dox fluorescence was efficiently quenched once loaded on the coordination polymer. The acidic microenvironment in lysosome could trigger Dox release and fluorescence recovery simultaneously, leading to real-time monitoring of drug release in tumor cells. The biocompatible dipeptide molecules (tryptophan-phenylalanine) could also coordinate with Zn²⁺ to form dipeptide nanoparticles, and the intrinsic fluorescence signal of the peptides shifted from the ultraviolet (UV) to the visible range due to π-π stacking interaction of aromatic groups and the structure rigidification by Zn²⁺ [63]. After being functionalized with MUC1 aptamer and Dox, the dipeptide nanoparticles could target cancer cells and image drug release in real time.

Polymeric nanovesicles obtained via self-assembly of amphiphilic small molecules hold a significant advantage in the ability to obtain a wide range of desired functions via the incorporation of hydrophobic/hydrophilic components with rational selection. A glycol nanovesicle (Lac-SS-DCM) is self-assembled by a rationally designed amphiphilic lactose derivative [59], in which a DCM moiety (used as the hydrophobic part and the fluorescence off-on probe) was pre-quenched and conjugated with a lactose derivative (used as the hydrophilic part and the targeting ligand to galectin-overexpressing tumor cells via enhanced carbohydrate-protein interactions) via a disulfide linkage (the GSH-responsive unit) (Figure 2B). The targeting Lac-SS-DCM nanovesicles were disassembled by GSH, and simultaneously activated the dormant...
NIR fluorescence of DCM, allowing the drug release triggered by the high GSH concentration in cancer cells and real-time monitoring of the release process.

For precisely controlling the drug release in vivo, DDSs with programmable release behaviors have been reported, which respond to multiple stimuli. Yan et al. [60] incorporated sequence-activated fluorescence responses into DDS to monitor multi-stimuli-triggered programmable drug release (Figure 2C). The smart AND logic dual-channel NIR fluorescent probe P(Cy-S-CPT) had a hierarchical structure composed of an ionizable tertiary amine-containing diblock co-polymer for rendering an ultrasensitive response to pH changes and a dual-channel NIR fluorescence component Cy-S-CPT for tracking the biothiol-triggered drug release. The programmable drug release of this hierarchical probe was conducted in a multi-stage tumor microenvironment, acidic endocytic organelles followed by abnormal GSH-overexpressing cell cytosol, which was synchronous with dual-channel NIR fluorescence output (830 and 650 nm, respectively). Impressively, in vivo fluorescence and 3D imaging demonstrated that P(Cy-S-CPT) could precisely target tumor tissue and perform controllable programmable drug release.

2.3 Mesoporous silica nanoparticle (MSN)-based drug release monitoring

To date, MSNs have become attractive delivery vehicles because of their unique structural features that enable high drug loading, ease in structure control, and versatility in functionalization. The ordered pore network within
these MSNs allows drug entrapping by diffusion. Meanwhile, the pores can be gated with various valves such as molecular motifs [64], nanoparticles [65–69], polymer multi-layers [70], DNA [71, 72], and proteins [73], which were designed to trigger the release of the entrapped drugs in the presence of multifarious external or internal stimuli. In light of numerous reports on the design and development of MSN-based stimuli-responsive DDSs, the development of strategies for real-time monitoring of drug release from MSNs is desired. Typically, optical agents were modified on the surfaces of MSNs or doped in the skeleton to track drug delivery and release in cells or tissues.

Lai et al. [64] designed a delicate molecular valve for the pores of MSN to control and monitor redox-responsive drug release (Figure 3A). The system comprised four components: (i) coumarin (FRET donor)-labeled-cysteine tethered MSNs as the drug carrier, (ii) fluorescein isothiocyanate (FITC, FRET acceptor)-attached β-cyclodextrin (β-CD) as the molecular cap to block the pores, (iii) disulfide linkage as the redox-responsive trigger to release the entrapped drug molecules, and (iv) FRET donor-acceptor for monitoring drug release in real time. Specifically, owing to linkage by the intact disulfide bond, the close proximity between coumarin and FITC resulted in FRET. In the presence of redox stimuli like GSH, the disulfide bond was cleaved, which led to the removal of the molecular cap (FITC-β-CD), thus triggering drug release and eliminating FRET. The release of the drugs from the MSN nanocarrier was monitored in real time following the changes in the FRET signal. It was also demonstrated that any exogenous or endogenous change in the GSH concentration would result in a change in the amount of released drug as well.

**Figure 3:** Monitoring drug release by fluorescent MSNs. (A) Schematic representation of the redox-responsive FRET-MSNs. (Adapted from Ref. [64].) (B) Microcarrier fate tracking and drug release monitoring in the NIR-II window using an InGaAs charge coupled device (CCD) camera. (Adapted from Ref. [74].) (C) Fluorescence lifetime imaging for drug release monitoring. (Adapted from Ref. [75].)
as concurrent change in the FRET signal, suggesting that the FRET-based MSNs could be applied to monitor the release of different drugs in real time.

By using SiO2 as template, Chen et al. [76] synthesized rare-earth (Tb, Eu)-doped Gd2O2S-based radioluminescent capsules with an X-ray-excited optical luminescence that changed during the release of the absorbing chemotherapy drug, Dox. In vitro experiments have demonstrated the pH-triggered release process of Dox from nanocapsules coated with a pH-responsive polyelectrolyte multi-layer. The Dox was loaded over 5% by weight and released from the capsule with a time constant of ~36 days at pH 7.4 and 21 h at pH 5.0, respectively. Other nanomaterials with specific functions are also combined with mesoporous silica to precisely control drug release. For example, metallic nanoparticles, such as gold and silver nanoparticles, have been widely explored for application in cancer diagnosis and therapies due to their fascinating properties. Gold nanorods (AuNRs) exhibit strong absorption and photothermal effect in the NIR region owing to their longitudinal surface plasmon resonance. Lee et al. [77] developed a NIR-responsive DDS based on mesoporous silica-coated AuNRs (AuNR@mSiO2) and phase change material (PCM). The AuNR core in AuNR@mSiO2 provided excellent NIR sensitivity and could efficiently quench the fluorescence of Dox, while the mesoporous silica shell provided an increased reservoir of the loaded drug (Dox). The PCM was applied as both a thermosensitive gatekeeper and a medium for loading hydrophobic anti-cancer drugs. The photothermal effect of the AuNRs under NIR irradiation could heat the PCM above its phase transition temperature and facilitate a rapid release of Dox and concurrently fluorescence recovery. An enhanced anti-cancer effect was observed against various cell lines, and the intracellular release of the drug was easily monitored by live cell imaging.

Shan et al. [78] covalently modified the mesoporous channel with the ligand thonyltribluorocetone firstly to in situ immobilize Yb+ ions, thus forming a Yb(III) complex. Then, the two-photon ligand 4,4’-bis[p-(diethylamino)-α-stryryl]-2,2’-bipyridine was used to coordinate with the central Yb+ ions to make the Yb(III) complex possess the NIR excitation (850 nm) and NIR emission (980 nm) property. Dox was then embedded into the mesoporous silica channel by coordination and π-π interaction with the Yb(III) complex, resulting in the decrease of photoluminescence intensity of the Yb(III) complex. Upon the drug release, the photoluminescence intensity of the Yb(III) complex became stronger because the coordinated Dox diffused out and the coordination sphere of the central Yb+ ions changed, thus achieving real-time drug release monitoring.

Compared to fluorescence imaging in visible range (400–750 nm) and the first NIR (NIR-I) window(750–900 nm) with low tissue penetration depth of several micrometers to millimeters because of severe absorption and scattering of photons by tissues, the second NIR (NIR-II) window (1000–1400 nm) features deeper penetration depths (1–2 cm) and higher resolution (sub-10 µm) with reduced scattering and minimized autofluorescence. Wang et al. [74] reported a novel microcarrier by integrating lanthanide-based nanoparticles (NaGdF4: 5%Nd@NaGdF4) emitting in the NIR-II window with MSNs (Figure 3B). The microcarrier realized in vivo semi-quantitative detection of the released drug in real time by measuring the fluorescence signals in the NIR-II window of lanthanide-based nanoparticles with an absorption competition-induced emission bioimaging system. The authors demonstrated that the microcarriers showed a prolonged residence time in the gastrointestinal tract up to 72 h and released up to 62% of the loaded drug. Moreover, minimal deposition of the microcarriers was found in non-target organs, such as the liver, spleen, and kidney. These findings provide novel insights for the development of therapeutic and bioimaging strategies.

Except for the fluorescence imaging relying on the intensity or spectral shift, fluorescence lifetime imaging is also a very attractive imaging tool for biomedical application, because it can reflect instantaneous local changes and allow distinguishing them from autofluorescence of the biological environment [79–81]. In view of these appealing features of fluorescence lifetime imaging, Valetti et al. [75] prepared label-free fluorescent mesoporous silica micro- and nanoparticles through in situ generation of carbon dots within the pores. By controlling the thermal decomposition of carboamino groups linked on the surface, three different types of mesoporous silica particles were synthesized: NLAB3, NLAB8, and NLAB14. The particles were endowed with emission in the visible region and had fluorescence lifetimes of up to 9.0 ns, which could be easily discriminated from intrinsic biological autofluorescence. The drug release was monitored successfully in biological environments by means of fluorescence lifetime imaging (Figure 3C).

### 2.4 Quantum dot (QD)-based drug release monitoring

QDs with the size of few nanometers are appealing candidates for nanodelivery and as optical probes in biomedical applications due to their excellent photostability, metabolic stability, and photoluminescence. Recently, several QDs such as carbon dots and silicon dots have been used...
to monitor drug release. Usually, the QDs were embedded into or capped onto nanocarriers to track the DDS and monitor drug release.

Carbon nanodots (CDots), owing to their high photostability, good water solubility, excellent biocompatibility, and multi-photon absorption, have emerged as a new class of fluorescence probes [82] and have also been extensively investigated for numerous applications, such as in medical imaging, in disease diagnosis, and as a print ink [83–85]. Tang et al. [86] reported a FRET-based CDot DDS (FRET-CDots) via directly coupling PEG onto the CDot surface followed by trapping the guest drug molecules within the PEG network and attaching to the CDot surface by π-π stacking, enabling real-time monitoring of drug release on the basis of the FRET signal (Figure 4A). In this case, the close proximity between CDots (serving as both excellent drug carriers and the donors) and Dox (the acceptor) made them form a FRET pair (FRET on). When the CDots were excited at 405 nm, emissions of both 498 and 595 nm were displayed, which correlated with the intrinsic emission of CDots and energy transfer from CDots to Dox, respectively. When Dox molecules are released from the CDot surface in the acidic environment, the FRET between CDots and Dox is terminated (FRET off), thus displaying an enhanced emission at 498 nm. This change in FRET signal could easily be regulated by the release of drug molecules from the CDot surface, thus allowing for convenient cell imaging and direct monitoring of the drug release process in real time. Moreover, the 3D two-photon imaging of the FRET-CDot at tumor tissues of 65–300 μm under the excitation of 810 nm suggested that the CDots had the potential to monitor drug release in deep tumor tissues.

Figure 4: Monitoring drug release by QDs. (A) Schematic representation of the FRET-CDot-DDS for drug delivery. Also, 3D two-photon confocal fluorescence images (under 810-nm excitation) of CDot-FA-Dox conjugate-incubated glomerular tissues, accumulated along the z-direction at a depth of 65–300 μm, incubated under pH of a) 7.4, b) 6.5, and c) 5.5. (Adapted from Ref. [86].) (B) Schematic representation of controlled release and real-time monitoring of the anticancer drug chlorambucil from ONBCbi-SiQDs. (Adapted from Ref. [87].) (C) Preparation and application of ATP-responsive GQDs-based nanocarriers. (Adapted from Ref. [88].)
Photoluminescent silicon QDs (SiQDs) have great potential in the area of fluorescence imaging owing to their unique properties, such as tunable emission, high natural abundance, low toxicity, and excellent surface tailorability [89], driving them be well explored as in vitro [90–92] and in vivo [93, 94] imaging agents. Recently, SiQD-based DDSs, which are responsive to pH changes, have been reported [95–97]. Paul et al. [87] used SiQDs as biocompatible fluorescent nanocarriers and o-nitrobenzyl (ONB) derivative as a phototrigger to construct a photoresponsive SiQD-based DDS (ONB-cbl-SiQDs) for the controlled release of the anti-cancer drug chlorambucil (Figure 4B). The ONB moiety with the caged anti-cancer drug chlorambucil quenched the fluorescence of SiQDs due to the photoinduced electron transfer process, which was termed as the fluorescence “off” state. Upon irradiation with one photon or two photons, uncaging of the drug occurred and led to the fluorescence recovery of SiQDs, considered as the fluorescence “on” state. This photoswitchable fluorescence signal was used to monitor drug release in real time.

Luminescent gold nanoclusters (AuNCs) consisting of several to tens of gold atoms have recently attracted significant scientific interest in fluorescence imaging. Importantly, the latest research showed that aggregated thiolate-AuNCs exhibited enhanced luminescence owing to the mechanism of AIE, and this phenomenon was closely connected to the restriction of intramolecular motion. Cao et al. [88] encapsulated aggregated AuNCs (aAuNCs) into metal-organic frameworks (MOFs, ZIF-8), which restricted the free movements of AuNCs in aggregated states, and thus enhanced their luminescence with an improved quantum yield of up to 7.74%. Once the acidic pH-induced dissociation of the ZIF-8 framework occurred, the aAuNCs were liberated from the MOF and dispersed in the form of individual nanoclusters with weaker luminescence. By encapsulating the hydrophobic drugs into the luminescent MOF simultaneously, the aAuNC-MOFs can achieve controlled release under acidic conditions and real-time imaging of pH-dependent drug release.

Graphene QDs (GQDs) maintaining the intrinsic layered structural motif of graphene have emerged as promising nanomaterials for fluorescence imaging and biosensing [99–102] due to their small lateral size, fine optical property, and superior biocompatibility. Our group has fabricated a smart drug nanocarrier based on FRET by capping GQDs (the acceptor) onto fluorescent MSNs (the donor) via ATP aptamer for real-time monitoring of ATP-triggered drug release [88] (Figure 4C). In this work, GQDs were used as both gatekeeper and quencher. By virtue of the sensitive interaction between ATP and ATP aptamer, any exogenous or endogenous fluctuation in the ATP concentration resulted in a change in drug release and concurrent variation in the FRET signal, which allowed real-time monitoring the release of drug from the pores.

2.5 Upconverting nanoparticle (UCNP)-based ratiometric luminescence for drug release monitoring

UCNPs are promising for bioimaging and biosensing owing to their minimized photodamage, suppressed autofluorescence, and high tissue penetration depth [103–108]. The unique ability of upconverting low-energy NIR light to high-energy UV, visible, and NIR light makes UCNPs an excellent nanotransducer for controlled drug release and an outstanding tracer for monitoring the drug release.

Wang et al. [109] coated a mesoporous SiO\textsubscript{2}/Ca layer onto UCNPs to form UCNPs@mSiO\textsubscript{2}-Ca core-shell nanocomposites, which were able to deliver anti-cancer drugs and monitor drug release. Generally, the NaYF\textsubscript{4}:Yb,Er UCNPs exhibited strong green emission (around 550 nm) and weak dark red emission (around 660 nm). Here, the incorporation of Ca into the nanocomposites induced phase transformation from a pure hexagonal phase to a cubic phase, and facilitated the occurrence of red emission, which strongly overlapped with the maximum absorbance of the anti-cancer drug zinc phthalocyanine to form a FRET pair. Consequently, drug release could be quantified by monitoring the luminescence recovery of UCNPs.

The simultaneous multiplex emission of UCNPs in the UV to NIR range is important for the design of multifunctional UCNP-based DDS: (i) the individual sharp emission peaks in the UV-visible region can serve as an energy donor for luminescence resonance energy transfer (LRET) to the entrapped drugs that typically absorb in UV-visible range, thereby allowing for real-time monitoring of the release of different drugs, whereas (ii) the NIR emission remains constant irrespective of the drug molecule and hence can be used for imaging or quantifying the drug release via a ratiometric signal. The conventional method to monitor the drug release using a single signal may be affected by an unknown local concentration of nanocarriers and uncontrolled microenvironment change in biological samples. Li et al. [110] presented a rational design for ratiometric monitoring of drug release kinetics in living cells by virtue of the multi-color emission feature of UCNPs (Figure 5A). In the system of mesoporous silica-coated NaYF\textsubscript{4}:Yb,Er nanocomposites loaded with Dox (NaYF\textsubscript{4}:Yb,Er@mSiO\textsubscript{2}-Dox), the green upconversion...
luminescence (UCL<sub>G</sub>) of the NaYF<sub>4</sub>:Yb,Er nanoparticles was selectively transferred to the drug Dox through the LRET mechanism, while the red upconversion luminescence (UCLR) was essentially unchanged and used as an internal standard. This ratiometric design employed red emission as the internal standard, and thus offered an accurate and reliable platform for localized drug delivery monitoring in living cells, avoiding the interference of the internal microenvironment and dosing errors. A linear relationship between the green-to-red emission intensity ratio (UCL<sub>G</sub>/UCLR) and the percentage of the released Dox was established.

Almost simultaneously, Lai et al. [111] utilized the multi-color UCNP with a core-shell structure of β-NaYF<sub>4</sub>:Yb<sup>3+</sup>/Tm<sup>3+</sup>@NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> to construct a ratiometric probe for monitoring drug release (Figure 5B). Such a core-shell structure of UCNP minimized the cross relaxation of activators, endowing the UCNP with bright emissions in the UV to NIR region (360, 475, 542, and 656 nm) when excited with a 980-nm NIR laser. This multi-color upconversion nanoparticle was firstly coated by mesoporous silica to form a UCNP@MSN composite nanoparticle. After being functionalized with zinc dipicolylamine analogue (TDPA-Zn<sup>2+</sup>), a compact branched polypeptide, poly(Asp-Lys)-b-Asp, was wrapped on its exterior surface through multi-valent interactions to entrap the drugs in interior mesopores. The UCNP emission in the UV-visible range was quenched due to LRET from the UCNPs to the entrapped drugs, while retaining their strong NIR emission. ATP had higher affinity to TDPA-Zn<sup>2+</sup> and competitively displaced the surface bound polypeptide, which led to the release of the entrapped drugs and subsequent collapse of LRET. The intracellular drug release was accompanied with the enhancement in the UV-visible range.
3 MRI for drug release monitoring

MRI is based on the spin-lattice relaxation (T₁ contrast) or the spin-spin relaxation (T₂ contrast) times of protons contained in different microstructures of the organs to create imaging contrast. As a powerful imaging modality in clinical medicine, MRI is a promising candidate for monitoring the biodistribution and site-specific accumulation of DDSs because it can produce non-invasive, tissue-depth-independent images with high spatial and temporal resolutions [112–114]. To improve its imaging sensitivity and resolution, paramagnetic ions, such as gadolinium (Gd³⁺) [115, 116], manganese (Mn²⁺) [117], and iron (Fe³⁺) [118], are typically used as MRI contrast agents to provide magnetic functionality. These ions can be intrinsic to the nanoparticles, incorporated as a dopant within a non-magnetic matrix, or attached to the surface of a nanoparticle via chelation [119]. Moreover, MRI is exquisitely suitable for monitoring drug release and drug efficacy. Because MRI contrast agents depend on access to freely diffusing water molecules to generate contrast, and therefore render different signals when drug molecules present within vs. outside of a nanocarrier, they provide optimal conditions for assessing drug release [120]. Recently, various nanoparticle-based contrast agents were developed to monitor drug release using MRI by right of the interaction between drug molecules and the MRI contrast agent. In particular, stimuli-responsive MRI nanosystems have been constructed typically in which magnetic resonance (MR) signals were turned off by forming complexes or encapsulated with drugs to shield the paramagnetic ions from the surrounding water. After triggering by endogenous or exogenous stimuli, such as pH, small molecules, temperature, or light, the MR signals show detectable changes along with the liberation or exposure of paramagnetic ions. These unique properties make MRI potentially applicable for drug release monitoring.

3.1 Gd-based MRI agents for drug release monitoring

As commercial MRI contrast agents, gadolinium chelates that can produce positive contrast enhancement in T₁-weighted MRI are beneficial for tumor diagnosis. Our group reported intelligent biodegradable DDS-based gadolinium-Dox-loaded nanoscale coordination polymers (Gd NCPs) for targeted drug delivery and programmed drug release [121]. As shown in Figure 6A, the gadolinium atoms not only acted as metallic nodes to conjugate the building blocks of 2-amino-1,4-benzenedicarboxylic acids (NH₂-BDC) to form uniform nanoparticles, but also as anchors to chelate Dox to form Gd³⁺-Dox complexes. Meanwhile, the gadolinium atoms in the core of the Gd NCPs were shielded well from water, making no contribution to T₁-weighted MRI contrast. By further coating a hyaluronic acid shell, the designed multi-functional nanocarriers were taken up especially by CD44-positive cancer cells via receptor-mediated endocytosis, and trapped within the lysosomes and endosomes. The acidic environment of lysosomes and endosomes caused the decomposition of the Gd NCPs into small fragments, along with the release of Dox. Therefore, water molecules could access the gadolinium atoms, manifesting as a “turned-on” T₁ signal for drug release monitoring. In vitro experiments showed that the Gd NCPs exhibited enhanced T₁ signal under acidic condition, suggesting their potential for localizing the tumor and monitoring drug release.

Kaittanis et al. [123] constructed a polymeric nano-beacon by chelating glucose-based polymer dextran with 89-zirconium and gadolinium. The positron emitter 89-zirconium could not merely image the tumor by positron emission tomography, but also guide the surgical resection of sentinel lymph nodes by their inherent Cerenkov luminescence. The MRI contrast agent gadolinium allowed monitoring the drug release with MRI through the quenching of the gadolinium signal by the co-loaded drug. Kim et al. [124] developed short-chain elastin-like polypeptide-incorporating thermosensitive liposomes (STLs) to co-encapsulate gadobenate dimeglumine (Gd-BOPTA) and Dox (Gd-Dox-STL). Gd-Dox-STL was highly stable under physiological conditions (37°C) and capable of rapidly releasing the encapsulated drug with mild hyperthermia (42°C) induced by high-intensity focused ultrasound (HIFU). Compared with lysolipid-based temperature-sensitive liposome loaded with gadolinium-based MRI contrast agent and Dox (Gd-Dox-LTSL), the Gd-Dox-STLs have higher stability and larger changes in T₁ relaxation time between 37°C and 42°C. In vivo MRI experiments demonstrated the co-release of Dox and Gd-BOPTA from STL under HIFU-induced mild hyperthermia, and thus the effectiveness of drug delivery and heating-triggered drug release could be assessed by detecting the MRI signals.
3.2 Mn-based MRI agent for drug release monitoring

The Mn-based agent is one kind of the earliest reported examples that can be used for enhancing $T_1$-weighted MRI owing to its efficient positive contrast enhancement. Cai et al. [122] constructed a smart Prussian blue-based theranostic nanoplatform with a core-shell hollow structure (namely HMPB-Mn) by coating an Mn-containing Prussian blue analogue (MnPBA) onto the outer surface and the inner channels of hollow mesoporous Prussian blue nanoparticles (HMPBs) (Figure 6B). The HMPB-Mn was highly sensitive to acidic pH and could release Mn$^{2+}$ at the tumor site, making HMPB-Mn a smart $T_1$-weighted MRI contrast agent with ultrahigh relaxivity for tumor diagnosis. Meanwhile, the hollow mesoporous structure and high surface area endowed HMPB-Mn with high drug (Dox) loading capacity for tumor chemotherapy. More important, both Mn$^{2+}$ ions and Dox molecules were released concurrently from the prepared HMPB-Mn in a pH-triggering manner, and the Dox release rate was real-time monitored through MRI. Combining the high-efficiency chemotherapy and excellent photothermal conversion property, HMPB-Mn also showed a great potential in synergistic chemothermal therapy.

Dong et al. [125] doped Mn$^{2+}$-chelated chlorine e6 [Ce6(Mn), a photosensitizer for photodynamic therapy] into mono-dispersed PEG-modified mesoporous CaCO$_3$ nanoparticles by co-precipitation. The as-synthesized mesoporous structure of Ce6(Mn)@CaCO$_3$-PEG nanoparticles could enable efficient loading of chemotherapeutic agent, Dox. With the degradation of the...
nanoparticles under weak acidic solutions, Ce6(Mn) and Dox were released attendant with the significantly enhanced $T_1$ contrast under MRI, making it possible to monitor the pH-dependent drug release in real time. Additionally, as guided by MRI and fluorescence imaging, it was found that the intravenously injected CaCO$_3@$Ce6(Mn)/Dox-PEG could gradually accumulate in the tumor and contribute to a synergistic anti-tumor effect in the combination of photodynamic therapy and chemotherapy.

### 3.3 Fe-based MRI agent for drug release monitoring

Although iron oxide nanoparticles as $T_2$ contrast agents have been widely used in MRI, they are rarely applied for drug release monitoring. Kaittanis et al. [126] reported that the clinical formulation of polymer-coated iron oxide nanoparticles (ferumoxytrol) could serve as a DDS in cancer chemotherapy (Figure 7). Specifically, the dextran coating retained diverse therapeutic payloads via weak electrostatic interactions. Once perturbed by mild acidification, the particles could rapidly release their cargo, which was utilized for the treatment of solid tumors. In addition to the improved therapy efficacy, the spatiotemporal drug release was monitored via MRI as the cargo release restored the spin-spin ($T_2$) and spin-lattice ($T_1$) relaxation signals of ferumoxytrol, while leaving the $T_1$ signature unchanged. In such a self-reporting multi-functional drug delivery platform, neither the particle nor the drug is subject to any chemical modification, which could lead to a faster administrative approval.

### 4 SERS imaging for drug release monitoring

SERS is an optical phenomenon in which the Raman scattering cross section can be increased by >6 orders of magnitude when the probe molecules are deposited on a roughened metal surface, which was first observed by Fleischmann in 1974 [127]. Since then, SERS has been widely used for biological sensing or molecular imaging as an ultrasensitive spectroscopic tool for interface studies [128]. The SERS method offers several advantages [129–132], including (i) ease of sample preparation, (ii) availability of characteristic peaks for label-free “fingerprinting,” (iii) narrow peak width for multiplexing detection, and (iv) good stability against photobleaching. Recently, Raman microscopy has made great progress in monitoring drug release. Some drug molecules such as thiopurine or mitoxantrone showed enhanced Raman scattering signal with the aid of gold nanostructures, which depends on the distance between the drug molecules and the nanoparticle surface. Therefore, the drug release process can be evaluated using the Raman signals of drug molecules, providing a promising method to monitor drug release in real time.

Thiopurine anti-cancer drugs can be adsorbed on the gold nanoparticle (Au NP) surface through its N and S atoms. Ock et al. [133] investigated GSH-induced drug release on Au NP surfaces by means of label-free confocal Raman spectroscopy (Figure 8A). Direct monitoring of GSH-triggered release of 6-mercaptopurine (6MP) and 6-thioguanine (6TG) was achieved in real time. The live cell imaging technique provides a signal from nanomolar range release of 6MP and 6TG on Au NP surfaces after the injection of external GSH. The in vivo SERS spectra of 6TG

![Figure 7: Monitoring drug release by Fe based MRI contrast agents.](image-url)
were obtained from the subcutaneous sites in living mice after GSH treatment. However, non-targeted therapeutic nanoparticles may cause harmful effects to healthy cells. In view of this, Hossain et al. [134] proposed an in situ label-free intracellular drug release monitoring system by using biohybrid nanoparticle based on SERS (Figure 8B). The biohybrid nanoparticle consisted of Au NP, cell-penetrating peptide (Tat peptide), and cancer-targeting antibody (anti-HER2 antibody) to obtain high anti-cancer efficacy with specific targeting and increased uptake rate. Both time-dependent and GSH-mediated intracellular release of Dox from the biohybrid nanoparticle was monitored with change of intracellular SERS signals of Dox. This proposed monitoring system made specific cancer cell targeting and the improved uptake of the anti-cancer drugs detectable, and the intracellular release of the anti-cancer drug visible.

Compared to Au NPs, gold nanostars with sharp-shaped metal surfaces display two surface plasmon bands consistent to the transverse and longitudinal surface plasmon bands in the visible and NIR regions, respectively. Tian et al. [135] proposed a new theranostic strategy based on efficient plasmonic-tunable Raman/FTIR spectroscopy imaging to track the delivery and release of an anti-cancer drug from nanostars in living cells and its biodistribution in vivo (Figure 8C). Mitoxantrone was conjugated onto the surface of gold nanostars with a carboxylated PEG spacer via amide bonding. By
taking advantage of this system, the delivery and release of mitoxantrone from gold nanostars in single living cells and in mice were tracked in real time, revealing a strong drug accumulation in the heart of healthy mice and infiltration in the tumor tissue of lung cancer-bearing mice at 5 h after systemic injection.

5 Multi-mode imaging for drug release monitoring

Although current imaging modalities show a lot of unique advantages, each of them also has its own intrinsic limitations, such as the insufficient spatial resolution of fluorescence imaging, difficult quantitative analysis of SERS imaging, and low sensitivity of MRI. These limitations make it difficult to gather accurate and reliable information at the disease site by using just one kind of imaging modality. To address these issues, there is an increasing demand for building up a complementary multi-mode imaging and monitoring method within one system.

5.1 Fluorescence- and MRI-based multi-mode drug release monitoring system

Liu et al. [136] reported a novel concept of real-time monitoring of NIR-triggered drug release in vitro and in vivo simultaneously by using upconverted luminescence (UCL) and MRI. Such a monitoring strategy featured the characteristics of high-sensitivity of UCL as well as high resolution and tissue depth independence of MRI. Concretely, multi-functional NaYF$_4$:Yb/Tm@NaGdF$_4$ core/hollow mesoporous silica shell nanoparticles (Gd-UCNP@hmSiO$_2$) were synthesized and applied in the dual-mode imaging nanosensor. Figure 9A illustrates the prototype nanosensor containing three essential components: (i) modified “photomechanical” azobenzene that acted as a “stirrer” on the mesopore surface of Gd-UCNP@hmSiO$_2$. 

![Figure 9: Multi-mode imaging for drug release monitoring.](image-url)

(A) UCL/T$_1$-MRI dual-mode nanosensor for drug release monitoring: confocal laser scanning microscopy images of zebrafish, in which UCNPs signals are green and Dox signals are red; T$_1$-MR images of a Walker-256 tumor-bearing Sprague-Dawley rat injected with nanosensor subcutaneously before and after NIR exposure for different time durations. (Adapted from Ref. [136].) (B) Schematic diagram of pH-triggered drug release tracking in acidic conditions by monitoring the SERS and fluorescence signal from the Dox molecules. (Adapted from Ref. [137].) (C) Mechanism of dual-mode monitoring of drug release and synergistic photothermal chemotherapy under a single NIR light. (Adapted from Ref. [138].)
photoluminescence signals of Eu\textsuperscript{3+} as TPNPs@cRGD). In the TPNPs@cRGD nanospheres, the anion (THA), and amine-functionalized cRGD (designated 4,4,4-trifluoro-1-(9-pentylenecarbazole-3-yl)-1,3-butanedione Gd\textsuperscript{3+}

to the surface of Au NPs, its SERS signal was enhanced upon drug release. In turn, once Dox was bound to the nanosensors, the fluorescence signal was quenched. In this field, the combination of fluorescence and Raman imaging has also been used for drug release monitoring. Kang et al. [137] presented a strategy based on plasmonic-tunable Raman/fluorescence imaging spectroscopy to track the release of an anti-cancer drug (Dox) from Au NP carriers at the single living cell level (Figure 9B). The pH-responsive drug release profile was attained through the conjugation of Dox to the nanoparticle surface via a pH-sensitive hydrazone linkage. When Dox was bound to the surface of Au NPs, its SERS signal was enhanced but its fluorescence was quenched. In turn, once Dox was released from Au NPs in acidic lysosomes, its Raman enhancement was greatly reduced and the Raman spectrum was changed; meanwhile, the fluorescence signal was recovered. The plasmonic-tunable Raman/fluorescence properties enabled the tracking of Dox release from the Au NP surface in living cells, and this system showed great potential in the investigation of the molecular mechanisms of drug delivery and release, as well as the cellular response to drug action.

Cao et al. [140] utilized a Janus particle with opposing mesoporous silica (MS) and gold faces for monitoring intracellular dual-drug (Dox and 6MP) release in real time based on FRET and SERS. The FRET acceptor Dox was attached to the donor 7-hydroxycoumarin-3-carboxylate (CMR)-conjugated MS with a pH-responsive linker hydrazone, and 6MP was conjugated to the gold surface through the gold-thiol interaction. The pH-triggered Dox release and GSH-responsive 6MP release were monitored according to the change of the CMR fluorescence signal and the decrease of 6MP SERS signal, respectively. This dual-drug system provided new perspectives for the real-time study of multi-drug delivery and release.

5.3 Fluorescence- and photothermal-based multi-mode drug release monitoring system

By utilizing drug-coordinated Eu\textsuperscript{3+} as a blocker to inhibit LRET from the Eu\textsuperscript{3+} complex to gold nanotriangles, multiple functions were integrated into one nanocomposite, such as operating under single NIR light, dual-mode monitoring of light-triggered drug release by photothermal imaging and two-photon luminescence imaging, and achieving synergistic photothermal chemotherapy [138] (Figure 9C). In this nanocomposite, Zhang et al. chose a two-photon-sensitized Eu\textsuperscript{3+} complex [Eu(THA),(phen)] as the LRET donor, gold nanotriangles as the acceptor, and a model drug (Dox) as the LRET blocker. The NIR light-mediated LRET enabled the dual-mode real-time monitoring of drug release as well as synergistic activation of multiple therapeutic potencies.

6 Conclusions and outlook

In this review, we discussed recent advances in drug release monitoring, including the strategies based on fluorescence imaging, MRI, SERS imaging, and multi-mode imaging. By comprehensively using a plethora of nanomaterials, these strategies could produce a signal response to drug release triggered by different stimuli, such as pH changes, enzymatic cleavage, redox, light, etc. Although a lot of advancements have been achieved in recent years, this field is still in its infancy and more research efforts are still desired in the future.

(i) At present, most drug release monitoring strategies are limited to qualitative determination. Developing new strategies for precise quantitative detection of drug release, especially in vivo, is urgently needed for accurately reflecting the relationship between drug release and treatment effect. Although many ratio-metric methods have been developed thus far, few
examples have had success in precise quantitative detection of drug release because their poor accuracy and precision are mainly hampered by the imaging sensitivity and resolution. In this regard, it would be helpful to construct novel functional nanoprobes with intriguing properties to improve the spatiotemporal resolution and sensitivity of imaging, improve the resolution of the instruments, or integrate multiple imaging contrast agents with different signal outputs.

(ii) The ability to complete multiple tasks, such as drug release monitoring, tumor microenvironment imaging, and treatment effect assessment, in one platform is desired for understanding the interaction among drug release, tumor microenvironment variation, and treatment effect. Developing nanomaterial-based contrast agents that possess smart, activatable, multi-responsive criteria is an appealing direction. For instance, if an agent can selectively change its generated signal amplitude or spectrum parameters along with different stimuli, it may respond to multiple biological events simultaneously. Alternatively, if the agent with different functional groups can be modified in response to different stimuli, the designed system can be used as an indicator of functional parameters to reflect various physiological/pathological status and visualize multiple events separately.

(iii) Considering that each imaging modality has its inherent pros and cons, the successful use of non-invasive imaging techniques is highly dependent on the choice of the right imaging modality and the right contrast agent. To accurately and quantitatively monitor the drug release, sometimes only one imaging modality is needed; however, in most cases, a combination of two or more different imaging techniques is required. Thus far, fluorescence imaging, MRI, and SERS are the most promising techniques, but the future exploration should not be limited to them. For example, there are some other burgeoning non-invasive imaging techniques, such as ultrasound imaging (USI), photoacoustic imaging (PAI), and optical coherence tomography (OCT), which hold great promise for monitoring drug release.

USI is based on the principle that the backscattered signals from acoustic waves vary depending on reflection by different tissues or ultrasound (US) contrast agents (i.e. gas- or air-filled micro/nanobubbles). US can convey a clear anatomical depiction of the tissue of interest, with high temporal and spatial resolution. In recent years, the US technique has been used for drug delivery by combining with microbubble- and nanobubble-based formulations, which can be either co-loaded with drugs or administered separately, to enhance extravasation, penetration, or cellular internalization and non-invasively track the fate of micro-/nanobubbles. Besides for imaging, US can also be used for therapeutic purposes by increasing the tissue temperature to 70°C for ablation with HIFU, or induce mild hyperthermia to change the carriers to release drugs with low-intensity.

PAI synergistically combines optical and USI technologies, and possesses high spatial resolution and deep tissue penetration. It is based on the photoacoustic effect that the optical absorption from non-ionizing laser pulses will be converted into heat, leading to transient thermoelastic expansion and thus wideband ultrasonic emission that is recorded by ultrasonic transducers to produce a 3D image. The emergence of various contrast agents such as organic chromophores (BHQ3, Cy5.5, Alexa 750, etc.) or inorganic nanoparticles (AuNRs, carbon nanoparticles, copper sulfide nanoparticles, etc.) with high NIR absorbance greatly accelerated the applications of PAI in tumor imaging and carrier tracking.

OCT can use the interference of light to generate two-dimensional cross-sectional images. Similar to USI techniques, OCT receives optical backscattered signal from the sample instead of an acoustic wave, enabling non-invasive acquisition of 2D/3D microstructures of biological tissue without extra contrast agents. In contrast, OCT can achieve a resolution as high as 1-10 μm, which is 1-2 orders of magnitude higher than that of USI, and a deep imaging at 2-3 mm compared with microscope-based techniques. Because of the features of non-invasive scanning, high imaging speed, and label-free imaging, OCT has been widely used in the biomedical fields, such as ophthalmology, cardiac diseases, dermatology, and cancer.

Although some of the techniques have been applied in clinical trials, their applications in drug release monitoring remains unexplored, possibly because of the poor resolution, high cost of the instruments, or lack of ideal imaging agents. Thus, it is highly promising to design multi-mode imaging agents using functional nanomaterials to make the most use of different imaging techniques, thus providing accurate, real-time, and in situ information to facilitate the translation of nanomedicine in treating human disease.

Acknowledgments: This research was supported by the Natural Science Foundation of Jiangsu Province (Funder Id: http://dx.doi.org/10.13039/501100004608, BK20180974, BK20170571) and the National Natural Science Foundation of China (Funder Id: http://dx.doi.org/10.13039/501100001809, nos. 21804059, 2170010150).
References


